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Transcription of *glnA* by purified *Escherichia coli* components: Core RNA polymerase and the products of *glnF*, *glnG*, and *glnL*

(glutamine synthetase/nitrogen regulation/σ factor)

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ABSTRACT **We have shown that the purified *glnF* (*ntrA*) product of *Escherichia coli* binds to core RNA polymerase. Together these proteins initiated transcription at the nitrogen-regulated promoter *glnAp2* on a supercoiled template. The initiation of transcription at *glnAp2* on a linear template required in addition NR_I, the product of *glnG* (*ntrC*), and $NR_{II}2302$, the product of a mutant allele of *glnL* (*ntrB*). These results identify the *glnF* product as a new σ factor specifically required for the transcription of nitrogen-regulated and of nitrogen-fixation promoters. We propose *rpoN* as the proper designation for *glnF*, and σ^{60} for its product. Our results indicate that σ^{60} RNA polymerase recognizes the nitrogen-regulated/nitrogen-fixation promoter consensus sequence C-T-G-G-Y-A-Y-R-N_4-T-T-G-C-A. Initiation of transcription in the intact cell appears to require in addition the active form of NR_I, the product of *glnG*. Conversion of NR_I to its active form is apparently brought about by NR_{II}, the product of *glnL*, in response to nitrogen deprivation.**

Escherichia coli and related bacteria possess a global control system that responds to nitrogen limitation. The genes and operons that are the target of nitrogen control encode proteins that permit the utilization of various alternative nitrogen sources when ammonia, the preferred source, is lacking in the growth medium. Examples of nitrogen-regulated genes include *glnA*, the structural gene for glutamine synthetase and the genes responsible for histidine utilization (1).

Extensive genetic analysis in several laboratories has revealed that activation of transcription of Ntr (*nit*rogen-*r*egulated) genes requires the products of *glnF* (*ntrA*) and *glnG* (*ntrC*) (1–5). Excess nitrogen appears to convert the *glnG* product to a form incapable of activating the transcription of these genes. The interconversion of the active and inactive forms of the *glnG* product is greatly stimulated by the product of *glnL*, which in turn receives an accurate assessment of the availability of cellular nitrogen from the products of *glnB* and *glnD* (1, 6–8).

The Nif (*ni*trogen-*f*ixation) genes of *Klebsiella pneumoniae* are under Ntr control, albeit somewhat indirectly. The *nif* locus consists of 17 genes organized into eight operons (9). One of these, the *nifLA* operon, encodes two Nif-specific regulatory proteins. During nitrogen-limited growth, transcription of *nifLA* is activated by the combined action of the products of *glnG* (*ntrC*) and *glnF* (*ntrA*) (10). The remaining *nif* operons are then similarly activated by the products of *nifA* and *glnF* (11–13).

The products of *nifA* and *glnG* share certain physical characteristics and may be evolutionarily related (14, 15). This relationship and the common requirement for the *glnF* product as coactivator strongly suggest that both activate transcription by the same mechanism.

All the Nif promoters and a few Ntr promoters have been sequenced and, in most cases, the transcripts have been mapped. Sequence comparison revealed that Nif promoters and Ntr promoters have great similarity to one another but no homology with a typical *E. coli* promoter (16).

The focus of the work in our laboratory has been the regulation of the complex *glnALG* operon. The *glnA* promoter region has recently been sequenced and two transcriptional-initiation sites have been identified (17–19). Promoter *glnAp1* is relatively weak and functions to provide low levels of glutamine synthetase during carbon-limited growth; it requires the catabolite gene activator protein (CAP) and cyclic AMP for activation and is repressed by NR_I, the product of *glnG*, in the presence or absence of the *glnF* product (19). On the other hand, *glnAp2* is an Ntr promoter by the criteria discussed above. It is a strong promoter responsible for providing high levels of glutamine synthetase during nitrogen limitation (19). We describe in this report the initiation of transcription at *glnAp2* in a purified system and the involvement of the products of *glnF*, *glnG*, and *glnL* in this process.

MATERIALS AND METHODS

General Methods. Protocols for plasmid isolation, plasmid construction, DNA sequencing, and nuclease S1 mapping were as described by Maniatis *et al.* (20).

Purification of NR_I. NR_I was isolated from *E. coli* strain TH19, which has a temperature-sensitive λ repressor and a deletion of *glnALG* (*glnALGΔ2000*), carrying plasmid pTH806. This plasmid contains *glnG* fused to the phage λ promoter P_L (21). The purification procedure was modified from that previously described (21).

It was found that addition of NaCl to 150 mM to the sonication buffer resulted in much greater recovery of NR_I. A heparin-Sepharose column was used in place of the phosphocellulose and DNA agarose columns, and fractionation on a hydroxyapatite column was included as a final step. The NR_I was judged to be at least 95% pure with no major contaminants as determined by visual inspection of a Coomassie blue stained $NaDodSO_4$/polyacrylamide gel.

Purification of the *glnF* Product. Strain W3110 $lacI^Q$/pTH7 (unpublished data), which carries *glnF* fused to the *tac* promoter (22) on a multicopy plasmid, was grown in 1 liter of LB medium (20), with ampicillin (25 μg/ml) at 37°C until a density of 150 Klett units (filter 54) was reached. Isopropyl β-D-thiogalactopyranoside (1 mM) then was added and the culture was incubated 4 hr. The culture was chilled and the cells were collected by centrifugation and resuspended in ≈30 ml of buffer S (50 mM Tris Cl, pH 7.8/1 mM dithiothreitol/10% (vol/vol) glycerol/1 mM EDTA). The cells were sonicated with a Branson cell sonifier and debris was removed by centrifugation. The supernatant was made 1% (wt/vol) in streptomycin sulfate, and the precipitate was re-

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Abbreviations: Ntr, nitrogen-regulated; Nif, nitrogen-fixation.
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moved by centrifugation. To the supernatant was added crystalline ammonium sulfate to 40% saturation. The pellet was resuspended in 25 ml of 20 mM imidazole, pH 7/1 mM dithiothreitol/10% glycerol containing enough NaCl to bring the conductivity to the equivalent of 200 mM and loaded on a 10-ml DEAE-Sephacel column. The column was washed with buffer until the eluate A_{280} was <0.1 above background. Then the bound material was eluted with a 100-ml linear 0.2–0.7 M NaCl gradient. A peak of the GlnF protein, identified by NaDodSO$_4$/PAGE, was eluted at ≈0.35 M. The pooled peak material was precipitated with ammonium sulfate (40% saturation) and resuspended in 5 ml of 10 mM Tris Cl, pH 8/100 mM NaCl/1 mM dithiothreitol/0.1 mM EDTA/10% glycerol and chromatographed on a 185-ml AcA 44 (LKB) column (1.5 × 100 cm). The pooled GlnF fraction was concentrated by adsorption to a 2-ml phenyl-Sepharose column and elution with 50 mM Tris Cl, pH 7.8/1 mM dithiothreitol/50% ethylene glycol. The protein was >95% pure as judged by inspection of a Coomassie blue-stained NaDodSO$_4$/polyacrylamide gel. The protein remained active for longer than 1 year when stored at −20°C.

Purification of the *glnL2302* Product. A plasmid overproducing the *glnL2302* product was constructed by crossing pgln94, which carries a promoter-up mutation at *glnLp* (23), with pgln43, which carries the *glnL2302* allele (7). Strain YMC21 carrying this plasmid, pTH819, was grown to saturation in 1 liter of glucose/glutamine minimal medium (24) at 30°C. The cells were spun down, resuspended in 30 ml of buffer S, and sonicated. The debris was spun down, and to the clear, amber supernatant streptomycin sulfate was added to 1%. The precipitate was pelleted and discarded. To the streptomycin supernatant, crystalline ammonium sulfate was added to 35% saturation. The precipitate was spun down and the supernatant was discarded. The pellet was extracted with 20 ml of 25%-saturated ammonium sulfate in buffer S. The insoluble material was spun down and the supernatant was discarded. The pellet was resuspended in enough 10 mM potassium phosphate/1 mM dithiothreitol/0.5 mM EDTA/10% glycerol to bring the conductivity down to ≈2 mS (around 50 ml), loaded on a 5-ml HA-Ultrogel column, and eluted with a 100-ml linear gradient from 20 mM to 500 mM potassium phosphate. The fractions containing the M_r 37,000 polypeptide (NR$_{II}$2302) were pooled.

The purified material was diluted with water to bring the salt concentration below 50 mM. The protein was then passed over a 2-ml heparin-Sepharose column. NR$_{II}$2302 runs through under these conditions. Our intent was to remove DNA-binding proteins and RNases that may have contaminated our preparation. The NR$_{II}$2302 was concentrated on a 5-ml DEAE-Sephacel column. We judge the purity of our NR$_{II}$2302 preparation to be 70–80%, with no major contaminants. The native protein appears to be a dimer.

Purification of RNA Polymerase. We purified RNA polymerase from 500 g of *E. coli* K-12 obtained (from Grain Processing, Muscatine, IA) as frozen, wet-packed cells. We used the method of Burgess and Jendrisak (25) except that we substituted a 50-ml heparin-Sepharose column (26) for the DNA-cellulose column. The enzyme was more than 50% saturated with σ^{70}. Core RNA polymerase was prepared by two cycles of chromatography on a Bio-Rex 70 column. The released σ^{70} was recovered and added back to a portion of the original holoenzyme preparation to make enzyme 100% saturated with σ^{70} (27).

Construction of Transcription Template pTH8. Plasmid pTE103 carries the bacteriophage T7 early terminator (28). A 600-base-pair *Hae* III fragment spanning the *glnA* promoter region (19, 29) and to which *Hin*dIII linkers had been added was inserted into pBR322 to generate pgln26 (unpublished results). The 600-base-pair fragment of pgln26 was then inserted into the unique *Hin*dIII site of pTE103 (Fig. 1). The assymetrically located *Sma* I site (29) was used to determine the orientation of the *glnAp*-bearing fragment with respect to the T7 terminator.

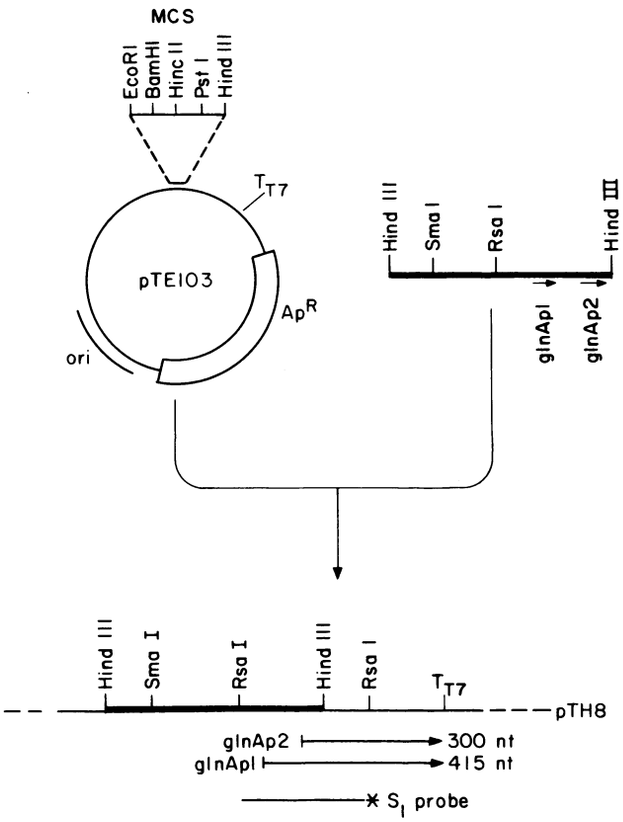

FIG. 1. Map of plasmid pTH8. The plasmid contains the *glnA* promoter region inserted into the multiple cloning site (MCS) region of pTE103. Transcriptional initiation at *glnAp1* and termination at T_{T7} should generate a transcript of ≈415 nucleotides (nt). Similarly, transcriptional initiation at *glnAp2* should generate a transcript of ≈300 nt. For nuclease S1-protection experiments, an *Rsa* I digest of pTH8 was end-labeled and the appropriate single-stranded probe was isolated (19, 20).

Transcription. Plasmid pTH8 was the template for all experiments. In those cases where linear DNA was used, the plasmid was first digested with *Eco*RI and reisolated by centrifugation in a CsCl gradient. *Eco*RI cuts pTH8 at the unique site shown in Fig. 1. The transcription buffer contained 50 mM Tris Cl (pH 7.8), 100 mM NaCl, 1 mM dithiothreitol, bovine serum albumin (200 μg/ml), 0.1 mM EDTA, and 10 mM $MgCl_2$. Reactions were carried out at 37°C in a total volume of 50 μl. ATP, GTP, and CTP were each present at 400 μM; unlabeled UTP was present at 40 μM and 20 μCi (1 Ci = 37 GBq) of [α-^{32}P]UTP was used per reaction. Template DNA was present at 20 nM, and RNA polymerase at 50 nM. NR$_I$ was added to 150 nM, NR$_{II}$2302 to 100 nM, and GlnF to 150 nM. All components except the UTP were added and the mixture was incubated at 37°C for 10 min. Heparin (Sigma) was then added to 200 μg/ml, followed immediately by the UTP. Reactions proceeded for 10 min and were stopped by adding 10 μg of yeast tRNA and 25 mM EDTA. The RNA was phenol-extracted, ethanol-precipitated, and subjected to electrophoresis in a 4% acrylamide/7 M urea gel.

RESULTS

Purification of the *glnF* Product. We constructed a plasmid on which *glnF* was fused to the strong *tac* promoter (22). The fusion was optimized to yield large amounts of the GlnF polypeptide (≈5% of cell protein) upon induction (unpub-

lished data). The details of the purification are found in *Materials and Methods*.

While working out a purification scheme, we noted the following properties of this protein: (*i*) it is acidic overall or has a highly acidic domain, inasmuch as it binds much more strongly to DEAE than the great majority of *E. coli* proteins; (*ii*) it does not bind phosphocellulose; (*iii*) it does bind heparin-agarose; (*iv*) it is monomeric as determined by gel filtration; and (*v*) its M_r estimated from its electrophoretic mobility on NaDodSO$_4$/PAGE (75,000) is greater than the M_r indicated by the length of the gene (60,000) (unpublished result). These physical properties are very similar to those of the RNA polymerase subunit σ^{70} (26, 27, 30).

The *glnF* Product Binds to Core RNA Polymerase. We added purified GlnF to core RNA polymerase and ran the mixture over a Bio-Gel A-1.5m column. As can be seen in Fig. 2, the peak of RNA polymerase contains significant amounts of the GlnF polypeptide. The constant ratio of this polypeptide to RNA polymerase in the fractions implies that they form a complex. Densitometric scanning of the gel gave ratios of 1:1:2:0.7 for $\beta/\beta'/\alpha$/GlnF polypeptides, consistent with the idea that the *glnF* product is a σ factor. The entire population of core polymerase molecules is apparently able to bind the *glnF* product.

The *glnF* Product Plus Core RNA Polymerase Alone Can Initiate Transcription at *glnAp2* When the Template Is Supercoiled. We used pTH8 (Fig. 1) as template for the experiments represented in Fig. 3. We found that initiation of transcription at *glnAp2* required only GlnF protein and RNA polymerase core enzyme (lane 2). Addition of the *glnG* product NR$_I$ had no effect at *glnAp2* (lane 3).

No initiation of transcription at *glnAp2* was observed when RNA polymerase charged with σ^{70} was substituted for the core enzyme (lanes 6 and 7). This observation indicates that σ^{70} and the GlnF protein bind to the same site on the core enzyme. We conclude therefore that GlnF plays the role of σ factor in the initiation of transcription at *glnAp2*.

Transcription was initiated at *glnAp1*, irrespective of whether our core enzyme was used alone or in combination with the *glnF* product or with the conventional holoenzyme. We assume that initiation of transcription at *glnAp1* requires σ^{70} and that our core polymerase preparation is slightly contaminated with σ^{70}. As expected, NR$_I$ repressed initiation at *glnAp1* (lane 3) (19).

When a linear template was used in place of the supercoiled template, no initiation of transcription at either *glnAp2* or *glnAp1* was observed (lanes 4 and 5).

Activation of Transcription at *glnAp2* by the Products of *glnG* and *glnL2302*. The failure of NR$_I$ to stimulate transcription could be ascribed to the inactive state of our preparation. Although, on the basis of genetic studies, the product of *glnL* is not required for the activation of transcription, it may mediate the conversion of NR$_I$ from an active to an inactive form and vice versa (8, 19). In the intact cell the product of a mutant, *glnL2302*, has been shown to maintain strong activation at *glnAp2*, irrespective of the nitrogen source of the growth medium (7). We reasoned that addition of this product to our system might stimulate the initiation of transcription at *glnAp2* on a linear template.

We have recently constructed plasmids that overproduce the products of *glnL* and *glnL2302* (unpublished work) and

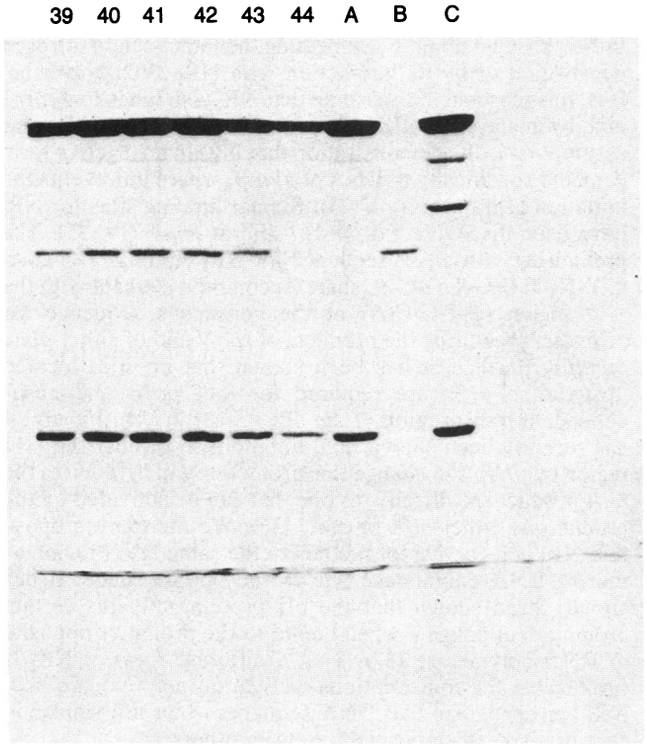

FIG. 2. Association of core RNA polymerase with *glnF* product. One milligram of RNA polymerase core enzyme was mixed with 0.3 mg of *glnF* product in 10 mM Tris Cl, pH 7.8/100 mM NaCl/1 mM dithiothreitol/0.1 mM EDTA/10% glycerol and incubated at 30°C for 10 min. The total volume was 2 ml. The protein solution was chilled to 4°C and loaded on a Bio-Gel A-1.5m column (1.5 × 100 cm) equilibrated in the same buffer. One hundred 1.8-ml fractions were collected. A portion was removed from selected fractions and the proteins were precipitated with trichloroacetic acid before addition of sample buffer and boiling. The proteins were separated by electrophoresis in NaDodSO$_4$ 7.5% polyacrylamide gel and stained with Coomassie blue. The lanes are labeled with fraction numbers; A (core RNA polymerase); B (*glnF* product); or C (holo RNA polymerase).

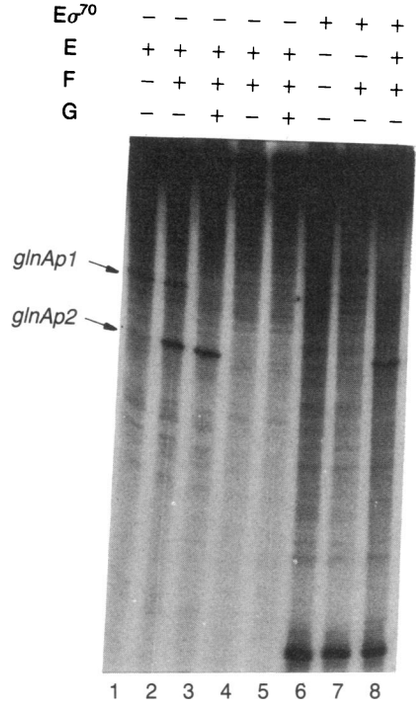

FIG. 3. Transcriptional initiation at *glnA* promoters. The experimental details are given in *Materials and Methods*. The figure shows the RNA products separated by gel electrophoresis. The composition of each reaction mixture is given above the corresponding lane. E, core RNA polymerase; Eσ^{70}, core RNA polymerase saturated with σ^{70}; F, *glnF* product; G, *glnG* product NR$_I$. The template was supercoiled (lanes 1–3 and 6–8) or linear (lanes 4 and 5).

have purified the product of *glnL2302* (*Materials and Methods*). We call this product $NR_{II}2302$.

The results of the experiments with $NR_{II}2302$ are shown in Fig. 4. It can be seen that core polymerase, GlnF, NR_I, and $NR_{II}2302$ are required for initiation of transcription at *glnAp2* on a linear template. Furthermore, we found that the initiation of transcription at *glnAp2* on the supercoiled template was strongly stimulated by NR_I together with $NR_{II}2302$ (compare lanes 1 and 3, Fig. 4).

Transcription Starts at the Same *glnA* Promoter Sites *in Vitro* and *in Vivo*. We prepared a single-stranded DNA probe spanning the *glnA* promoter region (see Fig. 1). This probe was protected from nuclease S1 hydrolysis by RNA synthesized in our purified transcription system. We determined the start site of transcription by the identification of the 5′ ends of the protected portion of the probe. The start sites were identical with those found in intact cells (19) (data not shown).

DISCUSSION

The results reported in this paper show that the initiation of transcription at *glnAp2*, the nitrogen-regulated promoter of the structural gene for glutamine synthetase of *E. coli* (19), requires only core RNA polymerase, the product of the *glnF* gene, and the four nucleotide triphosphates when the DNA template is in the supercoiled state. On the other hand, when the DNA template is linear, the initiation of transcription at *glnAp2* requires in addition NR_I, the product of *glnG*, and $NR_{II}2302$, the product of *glnL2302*, an allele of the normal *glnL* gene.

The results obtained when the linear template was used are in excellent accord with the results of genetic and physiological studies with intact *E. coli* cells. It has been found that the initiation of transcription at *glnAp2* requires the products of *glnF* and in addition either the product of *glnG* and a condition of nitrogen deprivation or the product of *glnG* and the product of the mutated *glnL* gene, *glnL2302* (19). Our present observation that NR_I is ineffective in the absence of $NR_{II}2302$ suggests that our purified system lacks the factor generated by subjecting the intact cells to nitrogen deprivation.

Because Ntr and Nif promoters differ so radically from the canonical sequence established for the majority of *E. coli* promoters, it has been suggested that they are recognized by their own specific σ factor (12). The physical properties of GlnF and in particular its ability to form a complex with core RNA polymerase (Fig. 2) identify this protein as a σ factor. The observation that GlnF fails to stimulate the initiation of transcription at *glnAp2* when RNA polymerase containing the normal σ^{70} is substituted for the core enzyme provides additional evidence for the view that GlnF substitutes for σ^{70} to initiate transcription at this promoter. To conform with the accepted nomenclature, *glnF* (*ntrA*) should be renamed *rpoN* (31). Although the exact molecular weight of the *rpoN* product has not yet been determined, the size of the gene suggests a molecular weight of 60,000 (unpublished observation) and we therefore propose to call it σ^{60} (32).

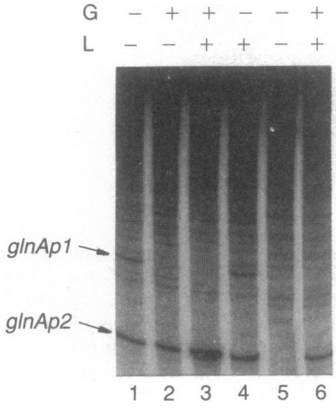

FIG. 4. Effect of $NR_{II}2302$ on transcriptional initiation at *glnAp2*. The figure shows the RNA products separated by gel electrophoresis. Each reaction mixture contained core RNA polymerase and the *glnF* product. The presence of the *glnG* product NR_I (G) and of *glnL2302* product $NR_{II}2302$ (L) is indicated above the respective lanes. The template was supercoiled (lanes 1–4) or linear (lanes 5 and 6).

With a supercoiled template, only σ^{60} RNA polymerase and the four nucleoside triphosphates are required for the initiation of transcription at *glnAp2*; this indicates that σ^{60} recognizes this promoter and, presumably, the promoters of the genes for the Ntr and Nif enzymes. Thus the consensus sequence for Ntr promoters (16),

$$\text{-C-T-G-G-Y-A-Y-R-N}_4\text{-T-T-G-C-A-N}_{6-11}\overset{+1}{\text{-N}}\text{-},$$

(where nucleotide +1 is the site of transcriptional initiation) may define the recognition site for σ^{60} RNA polymerase.

The σ^{60} RNA polymerase can apparently form a closed complex with the DNA of the Ntr promoters. The isolated plasmid DNA may have effectively greater superhelicity than it had in the intact cell (33) and the underwound state of this DNA may provide sufficient strand separation to permit transcription to proceed (34). The less supercoiled state of the cellular DNA and the relaxed state of the linear DNA may prevent the transition of the closed complex to the open complex unless NR_I activated by nitrogen deprivation or by interaction with $NR_{II}2302$ is also present. It is also possible that NR_I increases the affinity of σ^{60} RNA polymerase for the promoter and that the structural changes in the DNA that result from negative supercoiling also increase this affinity.

We are at present unable to explain the nature of the change in NR_I brought about by subjecting the intact cell to nitrogen deprivation or by its interaction with $NR_{II}2302$. Nevertheless, it is reasonable to assume that NR_I also binds to *glnAp2* and, by analogy, to other Ntr promoters. The reason for this assumption is the demonstration that NR_I in its inactive form can bind specifically to DNA at *glnLp*, where it prevents the initiation of transcription (23). Similar binding sites for NR_I have been discovered at *glnAp1* and at *dhuA* (19, 35). The preliminary consensus sequence for NR_I binding, T-G-C-A-C-Y-N_4-T-G-G-T-G-C-A, shares a common element with the −12 region (T-T-G-C-A) of the consensus sequence for promoters requiring the products of *rpoN* and of either *glnG* or *nifA*. Further, it has been shown that no sites located upstream of −30 are required for the *rpoN*- and *glnG*-dependent transcription of the *nifLA* operon (14). Finally, it has recently been shown that mutations in or near the −12 region of *nifHp* can change this promoter, which requires the *nifA* product specifically, to one that can be activated by the products of either *nifA* or *glnG* (36). We therefore propose that NR_I in its active form binds to the same DNA region as the σ^{60} RNA polymerase but on the opposite face. It has already been shown that the cII protein activates certain promoters of phage λ when bound to the promoter opposite to RNA polymerase (37). That a different form of NR_I is responsible for transcriptional activation may explain how NR_I can recognize two DNA sequences that are similar in certain respects yet very different in others.

It is of interest to consider the events initiating the transcription of the genes for heat shock proteins, which require σ^{32} (31). The rate of transcription of *rpoH*, the structural gene for σ^{32}, increases after heat shock. The increase in the cellular level of σ^{32} is then responsible, at least in part, for the activation of the transcription of genes that require σ^{32} RNA polymerase rather than the abundant σ^{70} RNA polymerase (31).

The *rpoN* product, σ^{60}, appears to be stable (unpublished observation), and judging from the level of β-galactosidase in cells with an *rpoN–lacZ*-operator fusion harboring a plasmid with a functional *rpoN* gene, the expression of *rpoN* is not increased by nitrogen deprivation (refs. 12 and 38 and

unpublished observations). These experiments also suggest that the intracellular level of σ^{60} is much lower than the level of the abundant σ^{70}. There is also evidence that the state of σ^{60} is invariant (13). It appears, therefore, that the cell contains at all times a subpopulation of σ^{60} RNA polymerase, perhaps bound to Ntr promoters in a closed complex. The trigger required to initiate transcription of these genes would then be NR_I in its active form. We postulate that, in cells growing with an excess of nitrogen, NR_I is inactive with respect to the initiation of transcription at *glnAp2* and similar Ntr promoters; that nitrogen deprivation brings about the conversion of NR_I to the active form by means of NR_{II}, the product of *glnL*; and that the resulting activation of transcription at *glnAp2* is responsible for an increase in the intracellular level of glutamine synthetase and of NR_I, both products of the *glnALG* operon (39). The increase in the level of NR_I, maintained in its active form by NR_{II}, would then result in the initiation of transcription of the other genes of the Ntr system (39). Transcription of these genes and of *glnA* would cease when, in response to nitrogen excess, NR_{II} converts NR_I back to its inactive form (19).

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1. Magasanik, B. (1982) *Annu. Rev. Genet.* **16,** 135–168.
2. Garcia, E., Bancroft, S., Rhee, S. G. & Kustu, S. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1662–1666.
3. Gaillardin, C. & Magasanik, B. (1978) *J. Bacteriol.* **133,** 1329–1388.
4. Kustu, S., Burton, D., Garcia, E., McCarter, L., McFarland, N. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4576–4580.
5. Pahel, G. & Tyler, B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4544–4548.
6. McFarland, N., McCarter, L., Artz, S. & Kustu, S. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2135–2139.
7. Chen, Y.-M., Backman, K. & Magasanik, B. (1982) *J. Bacteriol.* **150,** 214–220.
8. Backman, K. C., Chen, Y.-M., Ueno-Nishio, S. & Magasanik, B. (1983) *J. Bacteriol.* **154,** 516–519.
9. Roberts, G. P. & Brill, W. J. (1981) *Annu. Rev. Genet.* **35,** 207–235.
10. Buchanan-Wollaston, V., Cannon, M. C., Beynon, J. L. & Cannon, F. C. (1981) *Nature (London)* **294,** 776–778.
11. Sundaresan, V., Ow, D. W. & Ausubel, F. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4030–4034.
12. de Bruijn, F. J. & Ausubel, F. M. (1983) *Mol. Gen. Genet.* **192,** 342–353.
13. Merrick, M. (1983) *EMBO J.* **2,** 39–44.
14. Drummond, M., Clements, J., Merrick, M. & Dixon, R. (1983) *Nature (London)* **301,** 302–307.
15. Ow, D. W. & Ausubel, F. M. (1983) *Nature (London)* **301,** 307–313.
16. Ausubel, F. M. (1984) *Cell* **37,** 5–6.
17. Covarrubias, A. A. & Bastarrachea, F. (1983) *Mol. Gen. Genet.* **190,** 171–175.
18. Dixon, R. (1984) *Nucleic Acids Res.* **12,** 7811–7830.
19. Reitzer, L. J. & Magasanik, B. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1979–1983.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Reitzer, L. J. & Magasanik, B. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5554–5558.
22. deBoer, H. A., Comstock, L. J. & Vasser, M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 21–25.
23. Ueno-Nishio, S., Mango, S., Reitzer, L. J. & Magasanik, B. (1984) *J. Bacteriol.* **160,** 379–384.
24. Smith, G. R., Halpern, Y. S. & Magasanik, B. (1971) *J. Biol. Chem.* **246,** 3320–3329.
25. Burgess, R. R. & Jendrisak, J. J. (1975) *Biochemistry* **14,** 4634–4638.
26. Steinbach, H., Engelhardt, R. & Lezius, A. G. (1975) *Eur. J. Biochem.* **60,** 51–55.
27. Lowe, P. A., Hagen, D. A. & Burgess, R. R. (1979) *Biochemistry* **18,** 1344–1352.
28. Elliot, T. & Geiduschek, E. P. (1984) *Cell* **36,** 211–219.
29. Backman, K., Chen, Y.-M. & Magasanik, B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3743–3747.
30. Travers, A. A. & Burgess, R. R. (1969) *Nature (London)* **222,** 537–540.
31. Grossman, A. D., Erickson, J. W. & Gross, C. A. (1984) *Cell* **38,** 383–390.
32. Losick, R. & Pero, J. (1981) *Cell* **25,** 582–584.
33. Sinder, R. R., Carlson, J. O. & Pettijohn, D. E. (1980) *Cell* **21,** 773–783.
34. Richardson, J. P. (1975) *J. Mol. Biol.* **91,** 477–487.
35. Ames, G. F. & Nikaido, K. (1985) *EMBO J.* **4,** 539–547.
36. Ow, D. W., Xiong, Y., Gu, Q. & Shen, S. C. (1985) *J. Bacteriol.* **161,** 868–874.
37. Ho, Y. S., Wulff, D. L. & Rosenberg, M. (1983) *Nature (London)* **304,** 703–708.
38. Castaño, J. & Bastarrachea, F. (1984) *Mol. Gen. Genet.* **195,** 228–233.
39. Pahel, G., Rothstein, D. M. & Magasanik, B. (1982) *J. Bacteriol.* **150,** 202–213.